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POLYUNSATURATED FATTY ACIDS IN NORMAL HUMAN BLOOD

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Information on the fatty acid composition of human blood has been fragmentary and conflicting (1, 2). Wilson and Hansen (3) have reported unsaturated fatty acids in human plasma and suggested an average iodine number of 108. In 1937, Brown and Hansen (4) showed differences in the amounts of linoleic and arachidonic acids in sera of normal and eczematous children, and later Brown and others (5) demonstrated a decrease of plasma unsaturated acids in the adult man during a prolonged low fat diet. The lipide of human blood cells has been less extensively studied. Analyses by Erickson and coworkers (6) have shown that practically all blood cell lipide is in the stroma, mainly as phospholipide. Wiese and Hansen in 1953 (7) reported a semimicromethod for estimation of blood serum unsaturated fatty acids, but at that time did not possess constants for the pure natural acids and did not clearly characterize human blood lipides. The present availability of an adequate spectrophotometric method for the quantitative determination of polyunsaturated fatty acids suggested that a reinvestigation of human blood fats might be fruitful.

Methods

All subjects were young male medical students in the postabsorptive state (10 hours without food). From each of five subjects, 100 ml. of blood were drawn via the antecubital vein into heparinized syringes and immediately centrifuged. From two other donors a total of 910 ml. of whole blood was transferred by sterile technique to a sterile bottle to which had been added 240 ml. of a solution containing 3.17 gm. of sodium citrate, 1.15 gm. of citric acid, and 3.62 gm. of dextrose. After being mixed, 500 ml., containing 396 ml. of whole blood, were withdrawn immediately for extraction and analysis (Table I, Analysis 1). The remaining 514 ml. of blood were extracted and analyzed separately (Table

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I, Analysis 2). Neither the anticoagulant nor the amount of fatty acid extracted appeared to affect the analyses, and in our final evaluation all data were given the same weight. Plasma and blood cell fatty acids were extracted from each of the six blood specimens and were each analyzed in duplicate. In every case, plasma and cells were separated by centrifuging the whole blood specimens at 2000 r.p.m. for 1 hour. The plasma was pipetted off, and the cells were washed by resuspending and centrifuging them twice in mammalian Ringer's solution. Both plasma and blood cells were subjected to alcohol-ether extraction. Each was initially mixed with 95 per cent ethanol and heated to 60° under a partial vacuum to near dryness. The same procedure was repeated twice with ethanol-ether (3:1). The residue was transferred to a mortar, thoroughly ground with anhydrous sodium sulfate and ether, and filtered through a Büchner fun-

TABLE I

Comparative Analyses of Plasma Fatty Acids on Pooled Blood from Two Persons

The data, except iodine numbers, are expressed as percentages of total fatty acids.

Analysis No.	Linoleic acid	Linolenic acid	Arachidonic acid	Pentaenoic acid	Hexaenoic acid	Oleic acid	Total unsaturated acid	Total saturated acid	Iodine No.
1	22.72	1.85	5.43	0.91	1.21	36.2	68.3	31.7	106.2
2	23.85	2.57	5.73	0.91	1.28	33.2	67.5	32.5	108.8

nel. The processes of grinding and filtration were repeated six to eight times. The filtrate was evaporated first over a steam bath and then under reduced pressure to constant weight. After saponification, the unsaponifiable material was removed, and the fatty acids were extracted with petroleum ether by the method of Kerr and Sorber (8), as modified by Jamieson (9). After evaporation of the petroleum ether, the fatty acids were dried under a vacuum to constant weight and analyzed spectrophotometrically by the method of Herb and Riemenschneider (10). Iodine numbers were determined according to Wijs (11). At all times, except during the extraction and evaporation of solvent, precautions were taken to protect the samples from oxidation by keeping them under special oxygen-free nitrogen and storing them at 4°.

Calculations for the percentages of the individual polyunsaturated fatty acids were made according to Herb and Riemenschneider (10), as modified by Hammond and Lundberg (12) for the inclusion of hexaenoic acid. In the formulas used in the present work, the assumption was made that the pentaenoic acids consist of 50 per cent docosapentaenoic and 50

per cent eicosapentaenoic acids. The average differences between duplicate analyses of the fatty acids extracted from plasma and blood cells, in per cent, were as follows: linoleic 0.45, linolenic 0.18, arachidonic 0.31, pentaenoic 0.12, and hexaenoic 0.28. On the basis of these data and those of Table I, we believe our analyses for linoleic acid in plasma are accurate to

TABLE II

Fatty Acid Composition of Human Blood Plasma and Cells

The values for individual fatty acids are expressed as percentages of total fatty acids.

Subject	Whole blood	Total fatty acids	Iodine No.	Linoleic acid	Linolenic acid	Arachidonic acid	Pentaenoic acid	Hexaenoic acid
Plasma								
	ml.	*mg.*		*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
P. Y.	96	109	105.2	25.3	0.54	6.54	1.67	3.14
W. B.	104	106.7	114.5	26.2	0.85	7.82	1.31	2.08
R. M.	102	117.0	110.0	28.3	0.81	7.77	1.28	2.42
G. K.	122	98.4	108.8	25.9	1.21	7.47	1.75	2.46
P. C.	112	83.4	105.3	21.7	1.60	7.78	1.78	2.61
Average*.....			108.3	25.0	1.14	7.14	1.45	2.32
Cells								
P. Y.	96	23.1		6.62	−0.11	9.34	2.68	5.59
W. B.	104	97.3	95.6	6.09	−0.43	13.1	2.53	3.47
R. M.	102	62.8	97.7	8.09	−0.58	13.5	2.89	4.62
G. K.	122	85.6	98.7	9.29	0.52	11.8	2.73	3.13
P. C.	112	57.5	96.3	8.27	−0.17	14.3	3.16	4.22
W. Z. R. S.	396	285.4	115.4	8.68	−0.02	16.8	3.20	3.63
Average.....			100.7	7.84	0.00	13.1	2.87	4.11

* Includes data from Analysis 1, Table I.

within ±1 per cent, and are within a fraction of 1 per cent for any of the other plasma polyunsaturated fatty acids. Accuracy for blood cell fatty acids is probably of the same order.

RESULTS AND DISCUSSION

The percentage distributions of the individual fatty acids from blood cells and plasma are characteristically different, despite the fact that their iodine numbers are of the same order of magnitude (Table II). Of the poly-

unsaturated fatty acids, linoleic is predominant in plasma and arachidonic in blood cells. The small negative values for linolenic acid obtained in the analyses of blood cell fatty acids are probably within the limits of experimental error and indicate that essentially no linolenic acid is present. Pentaenoic and hexaenoic acids appear in both plasma and cells in more than trace quantities; to our knowledge there have been no quantitative analyses of these acids reported for human blood. The data for oleic acid are less precise than for the polyethenoid acids because of the difficulty in making accurate iodine number determinations on small fatty acid samples. For the same reason the values for total unsaturated and total saturated acids are less exact than those for the individual polyethenoid acids. Of the total plasma fatty acids, oleic acid averaged 21.1, total unsaturated acids 58.1, and total saturated acids 41.9 per cent. Of the total blood cell fatty acids, oleic averaged 11.3, total unsaturated 40.1, and total saturated 59.9 per cent.

Although the data obtained in these analyses are not extensive, we believe that they are of interest because they permit a more definite statement of the amounts and variability of individual blood polyunsaturated fatty acids than has hitherto been made. With blood levels approximately established, some new problems arise and numerous old ones present themselves for reexamination. The large amount of arachidonic acid in blood cells suggests that the formed elements of the blood may be a site for synthesis of this polyethenoid acid. In addition, the oxygen consumption of human serum *in vitro* in the presence of weak ammonium hydroxide and potassium ferricyanide, suspected by Litarczek (13) as being due to autoxidation of unsaturated fatty acids, might profitably be studied again.

SUMMARY

1. Spectrophotometric analyses have been made to determine the amounts of polyunsaturated fatty acids in the blood of seven normal human males in the postabsorptive state.

2. The data suggest that there is a characteristic distribution of polyethenoid acids in the fatty acids of both plasma and blood cells. Linoleic acid is the predominant polyunsaturated fatty acid in the plasma, whereas arachidonic acid is predominant in the cells.

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